

What is claimed is:

1. A recombinant non-cytopathic Rhabdovirus comprising a nucleic acid of a Rhabdoviral genome wherein said Rhabdoviral genome comprises a deletion or a mutation within a region encoding a matrix protein (M).
- 5 2. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising a deletion or a mutation within a region encoding a glycoprotein (G).
3. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising a regulatory element.
4. The recombinant non-cytopathic Rhabdovirus of claim 1, wherein said deletion
10 or mutation is in a region encoding the N-terminal half of said matrix protein.
5. The recombinant non-cytopathic Rhabdovirus of claim 4, wherein said deletion or mutation is in the region encoding the N-terminal part of said matrix protein encoding a nuclear localization sequence (NLS).
6. The recombinant non-cytopathic Rhabdovirus of claim 5, wherein said mutation
15 encodes for the substitution of:
 - (i) An alanine amino acid residue for a methionine amino acid residue, at position 33 or 51; or
 - (ii) A glycine amino acid residue for a serine amino acid residue, at position 226.
- 20 7. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising an insertion of a heterologous nucleic acid encoding a second polypeptide.
8. The recombinant non-cytopathic Rhabdovirus of claim 7, wherein said second polypeptide is a therapeutic polypeptide.
9. The recombinant non-cytopathic Rhabdovirus of claim 7, wherein said second
25 polypeptide is immunogenic.
10. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising an insertion of a heterologous nucleic acid encoding a marker polypeptide.

11. The recombinant non-cytopathic Rhabdovirus of claim 10, wherein said marker polypeptide is green fluorescent protein (GFP), secreted alkaline phosphatase, DS-Red fluorescent protein, beta-galactosidase, or luciferase.
12. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising an
5 insertion of a heterologous nucleic acid encoding a suicide gene.
13. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising an insertion of a heterologous nucleic acid encoding a cytokine gene.
14. The recombinant non-cytopathic Rhabdovirus of claim 13, wherein said cytokine is interleukin 2, interleukin 4, interleukin 12 or interferon- γ .
- 10 15. The recombinant non-cytopathic Rhabdovirus of claim 1, further comprising a Rhabdovirus G stem polypeptide.
16. The recombinant non-cytopathic Rhabdovirus of claim 1, wherein said recombinant non-cytopathic Rhabdovirus is being used as a gene delivery vector or a vaccine.
- 15 17. The recombinant non-cytopathic Rhabdovirus of claim 1, wherein said Rhabdovirus is preferentially cytopathic to neoplastic cells.
18. The recombinant non-cytopathic Rhabdovirus of claim 1, wherein said Rhabdovirus genome is a vesicular stomatitis virus (VSV) genome.
19. A method of producing a non-cytopathic recombinant Rhabdovirus comprising a
20 genetically modified nucleic acid encoding Rhabdovirus proteins including a deletion or a mutation within a matrix protein (M) comprising the steps of: (A) inserting into a suitable cell a polynucleotide sequence encoding Rhabdovirus proteins including a deletion or a mutation within the matrix protein (M), a polynucleotide sequence encoding a marker polypeptide and a polycistronic
25 cDNA comprising at least the 3' and 5' Rhabdovirus leader and trailer regions containing the cis acting signals for Rhabdovirus replication; (B) culturing the cell under conditions that select for a noncytopathic phenotype of said cell; (C) culturing said cell under conditions that permit production of the recombinant Rhabdovirus, and (D) isolating said non cytopathic recombinant Rhabdovirus.

20. The method of claim 19, wherein said non-cytopathic recombinant Rhabdovirus further comprises a heterologous nucleic acid sequence encoding a second polypeptide.
21. The method of claim 20, wherein said second polypeptide is a therapeutic polypeptide.
22. The method of claim 21, wherein said second polypeptide is immunogenic.
23. The method of claim 19, further comprising the step of isolating genomic RNA from said isolated non-cytopathic recombinant Rhabdovirus.
24. The method of claim 23, wherein said step of isolating genomic RNA is performed by using RT-PCR.
25. The method of claim 19, wherein said suitable cell, being selected from the group consisting of rodent, primate and human cells.
26. The method of claim 19, wherein said deletion or mutation resides in the N-terminal region of said matrix protein.
27. The method of claim 26, wherein said deletion or mutation residing in said N-terminal region of said matrix protein is part of a nuclear localization sequence (NLS).
28. The method of claim 19, wherein said mutation is an amino acid substitution of:
- (i) An alanine amino acid residue for a methionine amino acid residue, at position 33 or 51; or
 - (ii) A glycine amino acid residue for a serine amino acid residue, at position 226.
29. The method of claim 19, wherein the non-cytopathic recombinant Rhabdovirus is a vesicular stomatitis virus (VSV).
30. An isolated nucleic acid molecule comprising a polynucleotide sequence encoding a genome of a non-cytopathic Rhabdovirus, said polynucleotide sequence having a deletion or a mutation in a gene encoding a matrix protein (M).

31. The isolated nucleic acid molecule of claim 30, wherein said genome of a non-cytopathic Rhabdovirus further comprises a genetically modified glycoprotein (G).
32. The isolated nucleic acid molecule of claim 30, further comprising a regulatory element.
33. The isolated nucleic acid molecule of claim 30, wherein said deletion or mutation resides in a region encoding an N-terminal region of said matrix protein.
34. The isolated nucleic acid molecule of claim 33, wherein said deletion or mutation reside in the region encoding an N-terminal region of said matrix protein encoding a nuclear localization sequence (NLS).
35. The isolated nucleic acid molecule of claim 30, wherein said mutation encodes:
- (i) An alanine amino acid residue for a methionine amino acid residue, at position 33 or 51; or
 - (ii) A glycine amino acid residue for a serine amino acid residue, at position 226.
36. The isolated nucleic acid molecule of claim 30, further comprising an insertion of a heterologous nucleic acid sequence encoding a second polypeptide.
37. The isolated nucleic acid molecule of claim 36, wherein said second polypeptide is a therapeutic polypeptide.
38. The isolated nucleic acid molecule of claim 36, wherein said second polypeptide is immunogenic.
39. The isolated nucleic acid molecule of claim 30, further comprising an insertion of a heterologous nucleic acid sequence encoding a marker polypeptide.
40. The isolated nucleic acid molecule of claim 39, wherein said marker polypeptide is green fluorescent protein, secreted alkaline phosphatase, DS-Red fluorescent protein, beta-galactosidase, or luciferase.
41. The isolated nucleic acid molecule of claim 30, further comprising an insertion of a nucleic acid sequence encoding a suicide gene.

42. The isolated nucleic acid molecule of claim 30, further comprising a Rhabdovirus G stem polypeptide.
43. The isolated nucleic acid molecule of claim 30, wherein said genome is a vesicular stomatitis virus (VSV) genome.
- 5 44. A vector comprising the isolated nucleic acid molecule of claim 30.
45. A recombinant Rhabdovirus comprising a nucleic acid of a Rhabdoviral genome wherein said Rhabdoviral genome comprises a deletion or a mutation within a region encoding a membrane-proximal ectodomain of a Rhabdoviral glycoprotein (G).
- 10 46. The recombinant Rhabdovirus of claim 45, wherein said mutation encodes for the substitution of:
- (i) An alanine amino acid residue for a tryptophan amino acid residue.
 - (ii) An alanine amino acid residue for a glutamic acid, glycine and/or phenylalanine amino acid residue; or
 - 15 (iii) Aspartic acid and alanine amino acid residues for a glutamic acid, glycine or phenylalanine amino acid residue, or combinations thereof; or
 - (iv) Any combination of the substitutions in (a)-(c).
47. The recombinant Rhabdovirus of claim 45, wherein said mutation encodes for the deletion of:
- 20 (a) nucleotides encoding for the amino acid residues 449-461, or a fragment thereof; or
- (b) nucleotides encoding for the amino acid residues 440-449, or a fragment thereof.
- 25 48. The recombinant Rhabdovirus of 45, wherein said mutation is an insertion of the nucleotides encoding for the amino acid residues 311-319 of decay acceleration factor (DAF), inserted between serine amino acid residues of the Rhabdoviral glycoprotein membrane proximal ectodomain.

49. The recombinant Rhabdovirus of claim 45, further comprising an insertion of a heterologous nucleic acid sequence encoding a second polypeptide.
50. The recombinant Rhabdovirus of claim 49, wherein said second polypeptide is a therapeutic polypeptide.
- 5 51. The recombinant Rhabdovirus of claim 49, wherein said second polypeptide is immunogenic.
52. The recombinant Rhabdovirus of 45, further comprising an insertion of a heterologous nucleic acid sequence encoding a marker polypeptide.
53. The recombinant Rhabdovirus of claim 52, wherein said marker polypeptide is green fluorescent protein (GFP), secreted alkaline phosphatase, DS-Red fluorescent protein, beta-galactosidase, or luciferase.
- 10 54. The recombinant Rhabdovirus of claim 45, further comprising an insertion of a heterologous nucleic acid sequence encoding a suicide gene.
55. The recombinant Rhabdovirus of claim 45, further comprising an insertion of a heterologous nucleic acid sequence encoding a cytokine gene.
- 15 56. The recombinant Rhabdovirus of claim 55, wherein said cytokine is interleukin 2, interleukin 4, interleukin 12 or interferon- γ .
57. The recombinant Rhabdovirus of claim 45, further comprising a deletion or a mutation within the region encoding a matrix protein (M).
- 20 58. The recombinant Rhabdovirus of claim 57, wherein said genetically modified matrix protein comprises a mutation in an N-terminal region of said matrix protein.
59. The recombinant Rhabdovirus of claim 73, wherein said deletion or mutation in said N-terminal region of said matrix protein is part of a nuclear localization sequence (NLS).
- 25 60. The recombinant Rhabdovirus of claim 45, further comprising a regulatory element.
61. The recombinant Rhabdovirus of claim 45, wherein said recombinant Rhabdovirus is being used as a gene delivery vector.

62. The recombinant Rhabdovirus of claim 45, said recombinant Rhabdovirus is being used as a vaccine.
63. The recombinant Rhabdovirus of claim 56, wherein said Rhabdovirus genome is a vesicular stomatitis virus (VSV) genome.
- 5 64. A method of producing a recombinant Rhabdovirus comprising a genetically modified nucleic acid encoding Rhabdovirus proteins including a deletion or a mutation within a membrane-proximal ectodomain of a glycoprotein (G) comprising the steps of: (A) inserting into a suitable cell a polynucleotide sequence encoding Rhabdovirus proteins including a deletion or a mutation
10 within the membrane-proximal ectodomain of the glycoprotein (G), a polynucleotide sequence encoding a marker polypeptide and a polycistronic cDNA comprising at least the 3' and 5' Rhabdovirus leader and trailer regions containing the cis acting signals for Rhabdovirus replication; (B) culturing the cell under conditions that permit production of the recombinant Rhabdovirus,
15 and (C) isolating said recombinant Rhabdovirus.
65. The method of claim 64, further comprising the step of inserting a heterologous nucleic acid sequence encoding a second polypeptide into said cell.
66. The method of claim 64, wherein said second polypeptide is a therapeutic polypeptide.
- 20 67. The method of 64, wherein said second polypeptide is immunogenic.
68. The method of claim 64, further comprising the step of isolating genomic RNA from said isolated non-cytopathic recombinant Rhabdovirus.
69. The method of claim 68, wherein said step of isolating genomic RNA is performed by using RT-PCR.
- 25 70. The method of claim 64, wherein said suitable cell, being selected from the group consisting of rodent, primate and human cells.
71. The method of claim 64, wherein said mutation of a membrane-proximal ectodomain of the glycoprotein (G) encodes for the substitution of:
- (a) An alanine amino acid residue for a tryptophan amino acid residue.

(b) An alanine amino acid residue for a glutamic acid, glycine and/or phenylalanine amino acid residue; or

(iii) Aspartic acid and alanine amino acid residues for a glutamic acid, glycine or phenylalanine amino acid residue, or combinations thereof;
5 or

(iv) Any combination of the substitutions in (a)-(c).

72. The method of claim 64, wherein said mutation is a deletion of the:

(a) nucleotides encoding for the amino acid residues 449-461, or a fragment thereof; or

10 (b) nucleotides encoding for the amino acid residues 440-449, or a fragment thereof.

73. The method of claim 64, wherein said mutation is an insertion of the nucleotides encoding for the amino acid residues 311-319 of decay acceleration factor (DAF) inserted between serine amino acid residues of the Rhabdoviral glycoprotein membrane proximal ectodomain.
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74. The method of claim 64, wherein the non-cytopathic recombinant Rhabdovirus is a vesicular stomatitis virus (VSV).

75. An isolated nucleic acid molecule comprising a polynucleotide sequence encoding a genome of a Rhabdovirus, said polynucleotide sequence having a deletion or a mutation in a gene encoding a membrane-proximal ectodomain of the glycoprotein (G).
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76. The isolated nucleic acid molecule of claim 75, wherein said mutation of a membrane-proximal ectodomain of the glycoprotein (G), comprises substitution of:

25 (a) An alanine amino acid residue for a tryptophan amino acid residue.

(b) An alanine amino acid residue for a glutamic acid, glycine and/or phenylalanine amino acid residue; or

(iii) Aspartic acid and alanine amino acid residues for a glutamic acid, glycine or phenylalanine amino acid residue, or combinations thereof;
or

(iv) Any combination of the substitutions in (a)-(c).

5 77. The isolated nucleic acid molecule of claim 75, wherein said mutation is a deletion of the:

(a) nucleotides encoding for the amino acid residues 449-461, or a fragment thereof; or

10 (iii) nucleotides encoding for the amino acid residues 440-449, or a fragment thereof.

78. The isolated nucleic acid molecule of claim 75, wherein said mutation is an insertion of the nucleotides encoding for the amino acid residues 311-319 of decay acceleration factor (DAF) inserted between serine amino acid residues of the Rhabdoviral glycoprotein membrane proximal ectodomain.

15 79. The isolated nucleic acid molecule of claim 75, wherein said genome of a non-cytopathic Rhabdovirus further comprises a genetically modified matrix protein (M).

80. The isolated nucleic acid molecule of claim 75, wherein said deletion or mutation is in a region encoding the N-terminus of said matrix protein.

20 81. The isolated nucleic acid molecule of claim 80, wherein said deletion or mutation is in a region encoding a nuclear localization sequence (NLS).

82. The isolated nucleic acid molecule of claim 75, wherein said mutation encodes for the substitution of:

25 (a) An alanine amino acid residue for a methionine amino acid residue, at position 33 or 51; or

(b) A glycine amino acid residue for a serine amino acid residue, at position 226.

83. The isolated nucleic acid molecule of claim 75, further comprising a regulatory element.
84. The isolated nucleic acid molecule of claim 75, further comprising an insertion of a heterologous nucleic acid sequence encoding a second polypeptide.
- 5 85. The isolated nucleic acid molecule of claim 84, wherein said second polypeptide is a therapeutic polypeptide or is immunogenic.
86. The isolated nucleic acid molecule of claim 75, further comprising an insertion of a heterologous nucleic acid sequence encoding a marker polypeptide.
- 10 87. The isolated nucleic acid molecule of claim 86, wherein said marker polypeptide is green fluorescent protein, secreted alkaline phosphatase, DS-Red fluorescent protein, beta-galactosidase, or luciferase.
88. The isolated nucleic acid molecule of claim 75, further comprising an insertion of a nucleic acid sequence encoding a suicide gene.
- 15 89. The isolated nucleic acid molecule of claim 75, further comprising a fusion facilitating polypeptide or an antireceptor.
90. The isolated nucleic acid molecule of claim 75, wherein said genome is a vesicular stomatitis virus (VSV) genome.
91. A vector comprising the isolated nucleic acid molecule of claim 75.
- 20 92. A method for treating a subject suffering from a disease associated with a defective gene comprising the step of administering to a target cell of said subject a therapeutically effective amount of a recombinant non-cytopathic Rhabdovirus, wherein the genome of said Rhabdovirus includes a deletion or a mutation within a region encoding a matrix protein (M) and/or a and a heterologous gene capable of being expressed inside the target cell, thereby
- 25 treating the disease.
93. The method of claim 92, wherein said target cell is an epithelial cell, a lung cell, a kidney cell, a liver cell, an astrocyte, an immune cell, a glial cell, a prostate cell, or alpha, beta or delta cells of pancreatic islet, or acinar cells.

94. A method for immunizing a subject against a disease comprising the step of contacting a target cell of said subject with a therapeutically effective amount of a recombinant virus, wherein the virus comprises a Rhabdoviral genome, or fragment thereof, said Rhabdoviral genome or fragment thereof including a deletion or a mutation within a region encoding a matrix protein (M), and/or a deletion or a mutation within a region encoding the membrane-proximal ectodomain of a glycoprotein (G) and a heterologous gene encoding an immunogenic protein, or peptide fragment, capable of being expressed inside the target cell, thereby immunizing against a disease.
95. The method of claim 94, wherein said target cell is an epithelial cell, a lung cell, a kidney cell, a liver cell, an astrocyte, a glial cell, a prostate cell, a professional antigen presenting cell, a lymphocyte or an M cell.
96. A method for treating a subject suffering from a disease comprising the step of contacting a target cell of said subject with a therapeutically effective amount of a recombinant virus, wherein the virus comprises a Rhabdoviral genome, or fragment thereof, said Rhabdoviral genome or fragment thereof including a deletion or a mutation within a region encoding a matrix protein (M) and/or a deletion or a mutation within a region encoding the membrane-proximal ectodomain of a glycoprotein (G) and a heterologous gene encoding an immunogenic protein or peptide fragment, capable of being expressed inside the target cell, thereby treating a disease.
97. The method of claim 96, wherein said target cell is an epithelial cell, a lung cell, a kidney cell, a liver cell, an astrocyte, a glial cell, a prostate cell, a professional antigen presenting cell, a lymphocyte or an M cell.
98. A method for treating a subject suffering from a disease associated with a defective gene comprising the step of contacting a target cell of said subject with a therapeutically effective amount of a recombinant virus, wherein the virus comprises a Rhabdoviral genome, or fragment thereof, said Rhabdoviral genome or fragment thereof including a deletion or a mutation within a region encoding a matrix protein (M) and/or a deletion or a mutation within a region encoding the

membrane-proximal ectodomain of a glycoprotein (G) and a heterologous gene capable of being expressed inside the target cell, thereby treating the disease.

99. The method of claim 98, wherein said target cell is an epithelial cell, a lung cell, a kidney cell, a liver cell, an astrocyte, a glial cell or a prostate cell.

5 100. A method for cancer cell lysis, comprising the steps of contacting a cancerous cell with a recombinant Rhabdovirus, wherein said Rhabdovirus comprises (a) a nucleic acid comprising a Rhabdoviral genome, or fragment thereof, wherein said Rhabdoviral genome or fragment thereof comprises a deletion or a mutation within a region encoding a matrix protein (M) and/or a deletion or a mutation within a region encoding the membrane-proximal ectodomain of a Rhabdoviral glycoprotein (G); and (b) a non-Rhabdoviral nucleic acid.

101. The method of claim 100, wherein said non-Rhabdoviral nucleic acid encodes for a cytokine or suicide gene.

15 102. A method for treating cancer, comprising the steps of contacting a cancerous cell with a recombinant virus, wherein said virus comprises (a) a nucleic acid comprising a Rhabdoviral genome, or fragment thereof, said Rhabdoviral genome or fragment thereof comprises a deletion or a mutation within a region encoding a matrix protein (M) and/or a deletion or a mutation within a region encoding the membrane-proximal ectodomain of a glycoprotein (G); and (b) a non-Rhabdoviral nucleic acid.

103. The method of claim 102, wherein said non-Rhabdoviral nucleic acid encodes for a cytokine or suicide gene.

25 104. A method for identifying an agent that has oncolytic activity, comprising the steps of: obtaining vibrotome slices of corona, substantia nigra and cortex tissue, culturing said slices on coverslips under conditions maintaining viability and inhibiting mitosis, inoculating said slice culture with labeled cancer cells, culturing said inoculated culture with a candidate agent, and determining cancer cell viability, wherein a decrease in cancer cell viability indicates that the candidate agent is oncolytic, thereby identifying an agent that has oncolytic activity.

105. The method of claim 104, wherein said cancerous cells are of neuronal origin.
106. The method of claim 105, wherein said neuronal origin cancerous cells are glioma cells.
107. The method of claim 104, wherein said cancerous cells are labeled with a
5 fluorescent, luminescent, chromogenic or electron dense label.
108. The method of claim 104, further comprising the step of inoculating said slice culture with labeled recombinant Rhabdovirus.
109. The method of claim 104, further comprising the step of culturing said inoculated slice culture with a cytokine.
- 10 110. The method of claim 109, wherein said cytokine is an interferon.
111. The method of claim 104, wherein culturing said slices on coverslips under conditions maintaining viability is in a medium comprising Gey's/dextrose solution, plasma, thrombin, Eagle's basal medium, Hanks' balanced salt solution, L-glutamine, or any combination thereof.
- 15 112. The method of claim 104, wherein culturing said slices on coverslips under conditions inhibiting mitosis is in a medium comprising cytosine- α -D-arabinofuranoside, uridine, 5-fluoro-2'-deoxyuridine, Gey's/dextrose solution, plasma, thrombin, Eagle's basal medium, Hanks' balanced salt solution, L-glutamine or any combination thereof.